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PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

Improvements in or relating to Hepatic Medicines

We, BRACCO INDUSTRIA CHIMICA SOCIETA PER AZIONI, of Via E. Folli 50, Milan, Italy, a body corporate organised under the laws of Italy, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a new preparation suitable for use in the treatment of liver disorders, in particular chronic hepatitis, hepatosis (toxic degenerative damage to the liver) and cirrhosis. The novel composition according to the invention may also be used in the treatment of dietary disorders, such as protein deficiencies and protein resorption malfunctions caused by stomach or intestinal diseases and so on.

Nowadays, chronic liver diseases are mainly treated with corticosteroids, such as prednisone and prednisolone derivatives, or liver hydrolysates. The activity mechanism on which these treatments are based varies in accordance with the different types of preparations used, although the results are about the same. On average, the success factor is only from about 50% to at the most 60% of all the cases treated. Apart from their inadequate therapeutic activity, treatment wtih corticosteroids and treatment with liver hydrolysates may also produce undesirable side-effects. Corticosteroids may cause ulcer haemorrhages and various malfunctions of the metabolism and in particular, liver cell necrosis, which is shown by an increase in transaminases. Liver hydrolysates may also cause liver cell necrosis.

In view of these serious side-effects, safe therapy with corticosteroids and liver hydrolysates can only be carried out in clinics where the metabolism can always be checked.

Vitamin B 12 has recently been used in the treatment of liver diseases. It produces a symptomatic improvement in the general condition. Objectively, it is of course only possible with this therapy to stabilise the reduction in albumin and to increase the γ-globulin content in the serum of the liver patient.

A further improvement was provided by the combination of vitamin B 12 with folic acid.

In spite of this, these new preparations were not active enough either. This is attributable to the fact that neither vitamin B 12 nor folic acid in particular is able directly to influence and thus to regulate the affected functions of the metabolism. Physiologically, folic acid itself is totally ineffective. It is only the numerous conversion products of folic acid, in particular the tetrahydrofolates, which are directly able physiologically to influence the metabolism. Generally speaking, human tissue is only able to a very limited extent to convert folic acid into its reduced physiologically active metabolites (Robert *et al*, Proc. Amer. Assoc. Canc, Res. *4*, 57 (1963)). The already diseased, pathologically affected liver in particular is unable or at the most is very inadequately able chemically to convert folic acid into its physiologically effective secondary products, and therefore is hardly able to utilise folic acid in any way.

[Pr

Cf.:
1. *Moruzzi G, Marchetti M, Viviani R.*
"Effect of orotic acid on the synthesis of the ctirovorum factor and of methionine in chick liver"
(Nature *199*, 695 (1963)).

2. *Ferrari V.*
"La conversione dell'acido folico a "Citrovorum factor" nelle epatopatie sperimentali e cliniche"
(Acta, Vitaminol *9* 241 (1955)).

3. *Oji K., Wada M. Yoshida T.*
"Some aspects of the metabolism of the citrovorum factor in humans wtih liver disease"
(Med. J. Osoka Univ. *5*, 177 (1954)).

4. Kisliuk Medicine *43*, 711 (1964)

The inadequate activity of the preparation consisting of a combination of vitamin B 12 with folic acid is thus attributable to the unadequate synthesis of tetrahydrofolates by the diseased liver.

This failure is characteristic above all of serious liver diseases, decompensated cirrhoses in particular, which are becoming increasingly important in patient care. The present often unphysiological regimen of the modern individual which is characterised by an unsuitable food intake, frequently results in extremely serious disorders of the liver. In particular, the number of decompensated cirrhoses, which are usually attributable to alcoholism, is becoming increasingly larger.

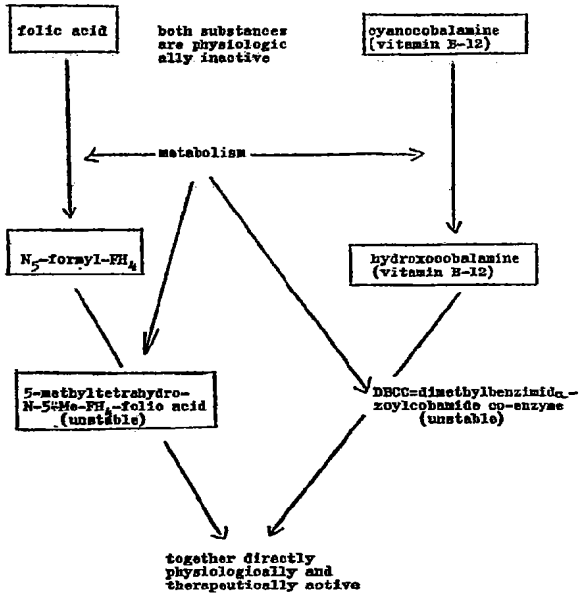
Until now, there have never been any really suitable preparations available for treating these disorders. It is an object of the present invention to fill this gap in therapy by means of a preparation which cures liver diseases, even in cases where conventional preparations have failed either completely or in part through inadequate activity.

This invention provides a therapeutic preparation suitable for use as a liver-protecting agent in the treatment of chronic hepatitic, hepatosis and cirrhosis and disproteinaemia, the preparation containing 5-formyltetrahydrofolic acid (N_5-formyl-FH_4) or a pharmaceutically acceptable salt thereof and vitamin B-12 as active components, wherein 75% of the vitamin B-12 consists of hydroxocobalamine and 25% of cyanocobalamine, and wherein the 5-formyltetrahydrofolic acid and vitamin B-12 components are used in an approximately equimolar ratio to one another which the 5-formyltetrahydrofolic acid component should not, or should only slightly exceed.

The percentages given are by weight.

The constituents of this preparation are directly required and used by the organism without any need for the organism to metabolise the substances fed to it in useful principles, which it would in any case be unable to do in its diseased state. Accordingly, the preparation of the present invention makes use of two necessary principles which, though stable enough for application, have little or no effect on the already pathologically weakened metabolism when developing their physiological activity in the organism.

The following highly simplified diagram shows the biochemical associations between N_5-formyl-FH_4 and vitamin B_{12} (cyanocobalamine and hydroxocobalamine). It is clear therefore that there is a close interrelationship in their effects. 5-methyl-tetrahydrofolic acid and DBCC coenzyme are unstable substances which cannot be stored so that 5-formyl-tetrahydrofolic acid and hydroxocobalamine represent the stable metabolic products.



The co-operation of both N_5-formyl-FH_4 and vitamin B12 is required to obtain the desired physiological effects required for successful therapy. Any B-12 deficiency blocks the enzymatic reactions of the tetrahydrofolate. For example, no methionine is produced where vitamin B-12 is deficient. The methionine-forming enzymatic metabolism reactions in which 5-formyl or 5-methyl tetrahydrofolic acid is required as a single-carbon transfer agent are stopped. Accordingly, the corresponding synthesis cycle is blocked, as is the protein synthesis, leading to the development of pernicious anaemia. Folic acid deficiencies based on this mechanism can be biochemically demonstrated for example in chronic parenchymatic malfunctions of the liver. Thus, due to the biochemical relationship, a B-12 deficiency also leads to folic acid deficiencies.

The same applies to the syntnesis of DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), for which the organism requires tetrahydrofolic acid compounds, i.e. tetrahydrofolic acid itself, 5-methyltetrahydrofolic acid or, preferably, the chemically stable 5-formyltetrahydrofolic acid, which are able directly to influence the biochemical synthesis cycle without affecting the metabolism in any way.

Accordingly, the liver-protecting preparation according to the invention is distinguished by the fact that it contains both 5-formyltetrahydrofolic acid and vitamin B-12 as its biologically active components and that 75% of the Vitamin B-12 is present in the form of hydroxocobalamine and the remaining 25% in the form of cyanocobalamine. Cyanocobalamine has a greater effect on the metabolism, giving a rapid onset of activity, whilst hydroxocobalamine remains in the blood for a relatively long period.

The folic acid component is preferably used in the form of calcium leucovorin (the calcium salt of *d*, l-L-5-formyltetrahydrofolic acid).

According to the invention, the 5-formyltetrahydrofolic acid and vitamin B-12 components should be used in approximately equimolar quantities. The folic acid content should not, or should only very slightly, predominate because under unfavourable conditions, an excess of the 5-formyltetrahydrofolic acid could use up essential natural vitamin B-12 reserves in the organism. The combination of 5-formyltetrahydrofolic acid and vitamin B 12 in approximately molecular proportions by weight is therefore necessary. The therapeutic effects of the novel preparation according to the invention are as follows: symptomatically, an increase in strength and energy and in the appetite, the disappearance of icterus, an increase in weight and an improved diuresis are observed in both chronic hepatitis and in cirrhoses.

In the case of liver punctures, a favourable change in the patholigical condition is obtained insofar as the disproteinacmia is improved. In the case of hepatitis, normal-

isation of the fat content is observed with the liver puncture, swelling of the liver is reduced and the appetite is increased. The positive effects observed in the case of decompensated cirrhoses are also particularly encouraging. Conventional therapeutic preparations were either inadequate or complete failures in this respect. Sustained therapy with the preparation according to the invention, which can be regularly administered over a period of years, also produces a histological improvement in the pathological condition.

The outstanding effect of the new preparation far exceeds expectations insofar as it is also effective in serious cases of hepatosis, chronic hepatitis and even in serious cases of compensated cirrhoses and in most cases of uncompensated cirrhoses which are cured either inadequately or not at all by conventional therapeutic treatments, particularly with regard both to the success factor and to the quality of the effects.

A comparison between the therapeutic results obtained with the compound preparation (A) according to the invention containing 5-formyltetrahydrofolic acid (N-5-formyl-FH_4) and vitamin B-12 and those obtained with the conventional compound preparation (B) containing corresponding quantities of folic acid and vitamin B-12, is given below on the basis of clinical tests carried out over an observation period of from 5 weeks up to 16 months (an average of 6 months).

Table 1 below demonstrates the variation both in the clinical symptons and in the laboratory findings.

Table 2 below shows the results of therapy with individual groups of patients.

Dosage and Administration:—

The method of treating liver disorders with preparations according to the present invention comprises regularly administering over a long period a composition consisting of individual doses of approximately 250—1000 γ-5-formyltetrahydrofolic acid, usually

TABLE I

Variation of the clinical symptoms and pathological laboratory conditions	Effect of preparation (A) 5-formyl FH_4+vitamin B-12 (according to the invention) No. of patients: 115			Effect of preparation (B) folic acid+vitamin B-12 (already known) No. of patients: 147		
	good	average	poor	good	average	poor
Improvement in appetite	83 (72%)	26 (23%)	6 (5%)	89 (61%)	32 (22%)	26 (18%)
Increase in physical strength and energy	79 (69%)	23 (20%)	13 (11%)	72 (49%)	46 (31%)	29 (20%)
Improvement in general condition	78 (68%)	19 (17%)	18 (16%)	65 (44%)	44 (30%)	38 (26%)
Reduction in icterus (from control of bilirubinaemia)	31 (63%)	11 (22%)	7 (14%)	21 (38%)	13 (24%)	21 (38%)
Increase in seralbumin	39 (34%)	63 (55%)	13 (11%)	53 (36%)	62 (42%)	32 (22%)
Increase in bromosulphophthalein storage as measured by the bromo-sulphophthalein test	53 (46%)	52 (45%)	10 (9%)	40 (27%)	68 (46%)	39 (27%)

Results: The above data show quite clearly the small number of failures with preparation (A) (average 11%) in comparison with the already known preparation (B) (average 25%).

TABLE 2

Illness	No. of patients	Treated with:—	Result	
			good	poor
Posthepatic syndromes	12	Preparation (A) Preparation (B)	11 (92%) 5 (56%)	1 (8%) 4 (45%)
Hepatoses	33	Preparation (A)	30 (91%)	3 (9%)
Chronic hepatitis	28	Preparation (A) Preparation (B)	22 (79%) 31 (65%)	6 (21%) 17 (35%)
Compensated cirrhoses	24	Preparation (A) Preparation (B)	18 (75%) 13 (68%)	6 (25%) 6 (32%)
De-compensated cirrhoses	18	Preparation (A) Preparation (B)	13 (72%) 14 (52%)	5 (28%) 13 (48%)

Explanation:
Preparation (A) = 5-formyltetrahydrofolic acid + vitamin B-12 (compound preparation according to the invention)
Preparation (B) = folic acid + vitamin B-12 (already known compound preparation)
On the result: The number of favourable results obtained with preparation (A) according to the invention is considerably greater than that obtained with the known preparation (B). In particular, the preparation (B) has a much greater failure quota.

in the form of the calcium salt of *d*, l-5-formyltetrahydrofolic acid and 500—2000 γ-hydroxocobalamine plus cyanocobalamine.

The superiority of the preparation (A) according to the invention is particularly pronounced in the most important and serious liver diseases, namely chronic hepatitis and cirrhoses.

In cases of decompensated cirrhoses, the failure quota with the new preparation (A) is only around 25%, whilst with the conventional preparation (B) it is approximately 50% of the cases treated.

The normal method of administering the novel liver-protecting preparation according to the invention is by intramuscular injection. The quantity specified below is usually injected daily.

In serious cases, injection is carried out twice daily.

For the treatment of less serious cases or for continuous treatment stretching over a period of six months or even years, an injection is administered every second day or twice weekly.

For oral therapy, 3 to 4 tablets or pills are required daily (see Example 3). Oral therapy is usually supported by injections. In the case of syrups (cf. Example 2), some 30 cc. (2 tablespoons) are administered daily. Similarly, this therapy may be supported by occasional injections.

EXAMPLES

1. Compositions of an individual dose intended for intramuscular injection consisting of:

(A) an ampoule containing

the calcium salt of d, 1-L-5-formyltetrahydrofolic acid	900 γ
Hydroxocobalamine hydrochloride (Vit. B 12)	1500 γ
Cyanocabalamine (Vit. B 12)	500 γ
and optionally	
D,L-inosine	60 mg.
Adenine	20 mg.
Nicotinamide	10 mg.
Sodium salt of riboflavin-5-monophosphate	2.73 mg.

Preparation: The substances listed above are dissolved in double distilled sterile water, the resulting solution is made up to a volume of 3 cc. per dose and filtered and sterile conditions, and the filtrate is introduced into ampoules (3 cc. per ampoule). The contents of the ampoules are lyophilised and the ampoules are sealed.

(B) A second ampoule containing:

double distilled water 2.5 cc.

and optionally

the sodium salt of D,L-acetylmethionine 50—55 mg.

Preparation: double distilled water optionally containing the sodium salt of acetyl methionine is filled into amopules and then sterilised.

Application: the ampoule B is opened. Its contents are taken up in a syringe and injected into ampoule A.

After the contents of ampoule A have been dissolved, the resulting solution is taken up in the syringe again and finally administered to the patient by intramuscular injection.

2. Composition of a syrup intended for oral administration.
A) Lyophilisate (in bottles) containing:

the Ca-salt of d,l-L5-formyltetrahydrofolic acid		1000 γ
hydroxocobalamine hydrochloride	Vitamin B-12	3000 γ
cyanocobalamine	Vitamin B-12	1000 γ
and optionally		
D,L-inosine		120 mg.
Adenine		40 mg.
Nicotinamide		20 mg.
Na-salt of riboflaving-5'-monophosphate		5.5 mg.
B) a syrup solution containing:		
methyl-*p*-hydroxybenzoate		80 mg.
propyl-*p*-hydroxybenzoate		40 mg.
Na-salt of D,L-acetylmethionine (optional)		100 mg.
Distilled water up to a volume of		120 cc.
Vermouth		42 c.c.
Syrup simplex		42 cc.

Preparation: —

A) The substances listed above are dissolved in distilled water, the resulting solution made up to a volume of 6 cc. per dose and filtered, after which the solution is poured into small bottles and lyophilised.

B) The substances listed above are dissolved in a little water, vermouth and syrup simplex being added to the resulting solution. The pH-value of the solution is adjusted to approximately pH 5 and the volume made up to 120 cc. The solution is then poured into bottles.

Application: Solution B is poured into bottle A with its lyophilised contents which are dissolved by shaking. The solution thus formed is administered orally to the patient. Orange essence may be used as a flavouring instead of vermouth.

3. Composition of tablets or pills:
An individual dose containing:

Ca-salt of d,l-L-5-formyltetrahydrofolic acid		100 γ
hydroxocobalamine hydrochloride	Vitamin B-12	400 γ
cyanocobalamine	Vitamin B-12	100 γ
and, as optional additives,		
D,L-methionine		20 mg.
D,L-inosine		15 mg.
adenine		5 mg.
nicotinamide		3 mg.
Na-salt of riboflavin-5-phosphate		1 mg.
and the excipients required to produce the tablets consisting of:		
starch		20 mg.
magnesium stearate		3 mg.
stearic acid		2 mg.
sodium metabisulphite		0.5 mg.
mannitol up to		120 mg.

The active substances are mixed with the excipients and the resulting mixture is made into tablets. The tablets may optionally be coated with acrylic or silicone resins by known methods.

WHAT WE CLAIM IS:—

1. A therapeutic preparation suitable for use as a liver-protecting agent in the treatment of chronic hepatitic, hepatosis and cirrhosis and disproteinaemia, the preparation containing 5-formyltetrahydrofolic acid or a pharmaceutically acceptable salt thereof and vitamin B-12 as active components, wherein 75% of the vitamin B-12 consists of hydroxocobalamine and 25% of cyanocobalamine, and wherein the 5-formyltetrahydrofolic acid and vitamin B-12 components are used in an approximately equimolar ratio to one another.

2. A preparation as claimed in claim 1, wherein the 5-formyltetrahydrofolic acid is in the form of the calcium salt of d,l-L-5-formyltetrahydrofolic acid (calcium leucovorin).

3. A therapeutic preparation as claimed in claim 1 substantially as herein described.

4. A therapeutic preparation as claimed in claim 1 substantially as herein described with reference to any of the specific Examples.

5. A process for the preparation of a liver-protecting therapeutic preparation wherein 5-formyltetrahydrofolic acid is mixed with at least an equivalent quantity of vitamin B-12, comprising 75% of hydroxocobalamine and 25% of cyanocobalamine.

6. A process for the preparation of a liver-protecting therapeutic preparation wherein the calcium salt of 5-formyltetrahydrofolic acid is mixed with at least an equivalent quantity of vitamin B-12 and the resulting product is lyophilised, 75% of the vitamin B-12 consisting of hydroxocobalamine and 25% of cyanocobalamine.

7. Therapeutic preparations prepared by a process as claimed in claim 5 or 6.

8. A therapeutic preparation for treating liver diseases, in unit dosage form, comprising 250—1000 γ of 5-formyltetrahydrofolic acid or the calcium salt of α,l-5-formyltetrahydrofolic acid and 500—2000 γ of 75% hydroxocobalamine plus 25% cyanocobalamine.

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